Chronic Thromboembolic Pulmonary Hypertension: Animal Models

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Short sentence:
This short review analyses strengths and weaknesses of all animal models available to study pathophysiology of CTEPH.
ABSTRACT

Chronic thromboembolic pulmonary hypertension (CTEPH) is a life-threatening disease due to pulmonary artery obstruction by persistent organised clots related to one or more episodes of acute pulmonary embolism. To date, the pathogenesis of CTEPH remains unexplained. Pulmonary endarterectomy removes obstruction from pulmonary vessels and can cure patients. However, some unreachable distal pulmonary obstruction and/or associated distal pulmonary vasculopathy could induce persistent pulmonary hypertension, the main postoperative complication. The pathophysiology of CTEPH is not fully understood and improving knowledge on this disease could improve our future surgical and medical management. Many attempts conducted over several decades have failed to reproduce this chronic disease in animals. However, several animal models have provided insights into the pathophysiology and pathogenesis of CTEPH. Here, we review all the animal models that have improved the comprehension of CTEPH and hold promise for further investigations.

Key words

Chronic thromboembolic pulmonary hypertension, remodelling, right ventricular function, pulmonary circulation
INTRODUCTION

Regarding the Dana Point 2009 Classification [1], chronic thromboembolic pulmonary hypertension (CTEPH) is a type 4 subtype of pulmonary hypertension (PH) in which pulmonary endarterectomy (PEA) [2] is effective in preventing death by right ventricle (RV) failure. CTEPH is due to obstruction of pulmonary arteries by persistent organised clots formed during one or more episodes of acute pulmonary embolism. The reason for clot persistence is unknown and the pathogenesis of CTEPH remains unexplained. To date, the lack of risk factors predicting the evolution from acute pulmonary embolism to CTEPH does not allow the development of preventive care and/or screening programs.

The increase in pulmonary vascular resistance is believed to result from a combination of proximal pulmonary artery obstruction and distal pulmonary vasculopathy [3,4], which have to be quantified before PEA to estimate risks and predict surgical success. However, the mechanisms underlying lesion development in the obstructed and unobstructed peripheral vascular beds remain unknown. Hence, medical management of inoperable CTEPH or operable CTEPH with an important distal vasculopathy remains troublesome because of a lack of efficient therapy.

To elucidate the pathophysiology of CTEPH, considerable effort has been expended in attempting to develop reliable animal models. Acute pulmonary embolism is easily produced in several animal species. In contrast, the induction of a disease replicating all the components of human CTEPH has proved challenging. These components consist of clot persistence and organization, pulmonary hypertension, chronic pulmonary-artery obstruction by unresolved intraluminal material, the development of a systemic blood supply to ischemic lung regions, pulmonary vasculopathy in unobstructed territories, and right ventricle
remodelling. Here, we review all the animal models described in the international literature and used to study the pathogenesis and pathophysiology of CTEPH (table 1).

**Animal models of persistent intravascular thrombosis**

The mechanisms by which the pulmonary emboli or thrombi fail to undergo lysis and instead organise into occluding fibrotic material remain unknown. Hypotheses include predisposing pulmonary-endothelial-cell abnormalities [5] and impairments in the vascular repair process [6]. A role for in situ thrombosis related to endothelial-cell dysfunction may explain why up to 63% of patients with CTEPH have no documented history of acute pulmonary embolism (3). This hypothesis has not been studied in animals, as no endothelial-cell abnormalities have been identified to date in humans with CTEPH. In contrast, the process of thrombus organisation has been studied in reliable animal models of low-flow-induced inferior vena cava thrombosis (table 2). Most of these animal models were developed in rodents, with the goal of investigating venous thrombus resolution [7-24]. Thrombosis can be induced by venous stasis alone or combined with induced blood hypercoagulability or mechanical endothelial damage. Kang and co-workers studied a piglet model of jugular vein thrombosis after stenosis and mechanical endothelial damage [15]. Inferior vena cava occlusion has been also studied in monkeys [17]. However, mice and rats remain the most widely used animals because of their better cost-effectiveness and efficiency [9]. Low-flow rodent models are characterised by a laminar thrombus that resolves within 3-4 weeks via a process of recanalisation that requires inflammatory-cell recruitment and angiogenic signals [7-13,19-24].

In studies of clinical risk factors, CTEPH was associated neither with the classical plasma-factor abnormalities associated with venous thromboembolism nor with impairments in fibrinolysis. However, having a ventriculo-atrial shunt or history of pacemaker infection
increased the risk of CTEPH [25,26]. This finding prompted studies in animal models. Thus, Bonderman et al. used a mouse model of low-flow venous thrombosis induced by inferior vena cava stenosis and endothelial damage to study the role for staphylococcal infection in delaying thrombus resolution and promoting the expression of profibrotic molecules [27].

**Dual vascular compartment theory**

Moser et al. [4] were the first to describe two compartments in the pulmonary vascular bed of CTEPH patients: an obstructed compartment subjected to chronic ischemia and an unobstructed compartment subjected to increased flow and shear stress (Figure 1). Thus, in addition to the obstruction of large pulmonary arteries, patients with CTEPH have vascular lesions in distal unobstructed territories. These distal lesions are similar to those found in patients with other forms of PH.

Many observations are consistent with a role for both territories in increasing pulmonary vascular resistance. First, for the same degree of pulmonary artery obstruction as measured by lung scanning, CTEPH is associated with higher pulmonary resistance values compared to acute pulmonary embolism [28]. Moreover, the onset of CTEPH disease is characterised by an asymptomatic honeymoon period during which pulmonary vascular resistance increases gradually without evidence of recurrent embolism. These two vascular compartments have been reproduced in animals separately then, very recently, simultaneously in a piglet model.

**Models replicating pulmonary artery obstruction**

The first attempt to replicate chronic pulmonary artery obstruction in an animal model was conducted in piglets by Fadel et al., who used proximal embolisation of coils and tissue adhesive into the left pulmonary artery [29]. Chronic obstruction (for 5 weeks) of the left
pulmonary artery resulted in chronic lung ischemia without PH. The result was an increase in the systemic blood supply to the lung via bronchial, mediastinal, and intercostal arteries, as well as distal post-obstructive pulmonary vasculopathy. These lesions were similar to those seen after chronic pulmonary artery ligation [30].

The left pulmonary artery was chosen for ligation, because it could be easily re-implanted into the main pulmonary artery to replicate reperfusion injuries after PEA. The systemic vascular response to chronic pulmonary vascular obstruction differs across species, with proliferation of bronchial arteries into the intraparenchymal airways in large animals (dogs and piglets) and rats or of intercostal arteries into the pleural space in mice [31]. Although CTEPH studies have been conducted chiefly in piglets, a few studies of bronchial circulation have been performed in a rat model of pulmonary artery ligation [32]. Occlusion of one of the main pulmonary arteries stimulates angiogenesis in the bronchial vessels of the ipsilateral lung [33]. Bronchial arteries begin to enlarge as soon as 2-3 days after pulmonary artery ligation and supply the pulmonary circulation via precapillary anastomoses. These anastomoses may maintain airway epithelium oxygenation, thus explaining the lesser degree of reperfusion injury after chronic lung ischemia than after acute lung ischemia [34].

In addition to bronchial circulation hypertrophy, post-obstructive vasculopathy is characterised by pulmonary artery abnormalities including increased media thickness [35], impaired vasoreactivity [36], impaired endothelial nitric-oxide-synthase function [35], and increased reactivity to endothelin-1 (ET-1) [37] leading to increased resistance after reperfusion [38].

Lung reperfusion after chronic ischemia was followed by gradual reversal of the post-obstructive vasculopathy [30, 39], after an early phase of ischemia-reperfusion injury with endothelial-cell damage [40].
Models replicating lesions of unobstructed pulmonary arteries

During the early phase of CTEPH, the unobstructed territories are subjected to a chronic blood-flow increase due to cardiac output redistribution. Therefore, systemic-to-pulmonary shunts have been used in animals to replicate the lesions seen in unobstructed territories in patients with CTEPH [41]. In an animal model initially developed by Rendas et al. [42] to replicate congenital heart disease, an aorto-pulmonary shunt is induced by implanting a short prosthesis between the ascending aorta and main pulmonary artery. This model has been used to induce high-flow pulmonary vascular lesions similar to those seen in unobstructed territories in CTEPH (Figure 2). As with the left pulmonary-artery ligation models, the aorto-pulmonary shunt was achieved through a median sternotomy to avoid pleural opening and a subsequent inflammatory response. High-flow pulmonary vasculopathy induced by 5 weeks of aorto-pulmonary shunting was characterised by increased media thickness of the distal pulmonary arteries (Figure 3) related to smooth-muscle-cell proliferation (as shown by proliferating cell nuclear antigen labelling) and by elevated levels of ET-1 and its receptor ETA in lung tissue. ET-1 is a potent vasoconstrictor and mitotic peptide for vascular smooth muscle cells. ET-1 overexpression has been found in other animal models of high pulmonary flow [43-45]. ET-1 overproduction is probably a response to stimuli such as shear stress resulting from arterial pressure elevation [46].

Closure of the aorto-pulmonary shunt replicates the hemodynamic conditions in unobstructed territories after PEA. Shunt closure induced normalisation of ET-1 and ETA expression, followed by media hypertrophy reversal in the distal pulmonary arteries. These findings were consistent with the gradual improvement in pulmonary vascular resistance observed 3 to 6 months after PEA.
**Animal model replicating all features of CTEPH**

Although the above-described animal models provided useful information on impaired thrombus resolution and on the pathophysiology of lesions in obstructed and unobstructed territories, they failed to replicate important features of human CTEPH including pulmonary hypertension, interactions between the two pulmonary vascular compartments and, above all, RV remodelling and dysfunction.

Since the 1990s, several attempts to develop animal models of CTEPH [47-51] failed because of clot lysis by the very efficient endogenous fibrinolytic system [52] and of the remarkable adaptive capabilities of the pulmonary circulation. Thus, 3 hours after acute pulmonary embolism in dogs [47], only 30% of the initial injected thrombus volume remained inside the pulmonary artery. Adding tranexamic acid [48] or plasminogen activator inhibitor-1 [49] to delay thrombus resorption between injections failed to solve this problem. The second difficulty was the large pulmonary circulation reserve, which required obstruction of more than half the pulmonary vasculature to achieve an increase in pulmonary vascular resistance. The adaptive capabilities of the pulmonary vasculature explain why repeated injections of small, inert, non-absorbable materials failed to replicate CTEPH. Thus, in a dog model of chronic pulmonary-artery injections of 3 mm in diameter ceramic beads, pulmonary pressures and resistances returned to normal within 1 week after each injection [50]. With 100-300 µm microspheres, 60 days of repeated embolisation were required to increase the pulmonary-artery pressure [51]. After 60 days, signs of PH started to develop, but there was no bronchial artery hypertrophy, post-obstructive or high-flow vasculopathy, or proximal vascular obstruction [51]. A third challenge lies in replicating RV remodelling. Extensive acute obstruction of the pulmonary arterial tree is usually lethal by heart failure in the absence of previous RV training and hypertrophy. To tolerate a persistent increase in pulmonary arterial pressure, the RV must undergo remodelling, which consists in
gradual ventricular wall hypertrophy followed by right-heart-chamber enlargement with a paradoxical septal motion. In the event of persistent pulmonary hypertension, RV failure develops.

We recently developed a CTEPH piglet model [53] consisting in primary left pulmonary-artery ligation via a sternotomy followed by weekly transcatheter embolisation, under fluoroscopic control, of the tissue adhesive enbucrilate (Histoacryl®) into the right lower lobe for 5 weeks. Pulmonary artery ligation overwhelmed the pulmonary circulation reserve and led to PH within a few weeks, with progressive obstruction of the remaining lung vasculature. The progressive nature of the obstruction achieved via weekly embolisation allowed the RV to adapt to the pressure increase, thus preventing death by acute RV failure. The tissue adhesive enbucrilate solidifies immediately after contact with blood and adheres to the arterial wall. The result was proximal obstruction by unresolved material in the right lower-lobe artery. Thus, the right upper-lobe arteries remained patent and exhibited lesions replicating those seen in unobstructed territories in CTEPH. After 5 weeks, this piglet model replicated all the features of human CTEPH: increased pulmonary vascular resistance, increased mean pulmonary artery pressure, increased media thickness of distal pulmonary arteries in both obstructed and unobstructed territories, increased systemic blood supply through the bronchial arteries in the obstructed territories, RV hypertrophy, RV enlargement, and paradoxical septal motion (Figure 4). Interestingly, although the embolisations were stopped after 5 weeks, the increase in pulmonary vascular resistance persisted for up to 1 month later after the last embolisation [personal unpublished data]. Overexpression of ET-1 and its receptors ETA and ETB was documented in the remodelled distal arteries of the unobstructed territories, in keeping with previous findings in piglets with high-flow vasculopathy [41,43]. In addition, ETA overexpression in the obstructed territories was consistent with the results of previous studies of post-obstructive vasculopathy [38]. Further
experiments are needed to monitor pulmonary resistance at a distance from the last pulmonary embolisation, to investigate RV remodelling and failure, and to assess interactions between the obstructed and unobstructed territories. However, one should bear in mind that this model does not replicate the impaired thrombus resolution seen in human CTEPH.

**Conclusions**

Animal models developed over several decades have provided valuable information on the pathophysiology of CTEPH. Thrombus resolution and obstructed and unobstructed territories have been studied separately in several animal models. Recently, a model replicating all the major aspects of human CTEPH was developed. This model should prove useful for investigating RV dysfunction and distal lung vessel abnormalities. However, additional models are still needed to elucidate the pathobiology of thrombus persistence.
REFERENCES


LEGENDS

**Figure 1:** Anteroposterior pulmonary angiogram showing the two vascular territories in a patient with CTEPH: the obstructed territory is subjected to chronic ischemia and the unobstructed territory to an increase in blood flow.

**Figure 2:** Aortography showing the aortic arch with the two supra-aortic arteries in a sham-group piglet. (A) Visualisation of the pulmonary vascular bed (white arrow) at the same time as the aortic arch after creation of an aorto-pulmonary shunt. (B) No opacification of the pulmonary vascular bed after shunt closure (C).
Figure 3: Box plot of media thickness percentage of small pulmonary arteries (<150 μm) in the sham (35.9±0.8%), shunt-open (55.6±1.2%), 1-week shunt-closed (48.7%±1), and 5-weeks shunt-closed (40.9%±1) groups (from [41]). Media thickness percentage (MT%) was calculated as follow: MT%=(ED-ID)/ED where ED was external diameter and ID internal diameter. Aorto-pulmonary shunting was created by a graft interposition between the ascending aorta and the pulmonary trunk in piglets. Closure of the shunt was obtained by dividing the graft.
**Figure 4:** Chronic thromboembolic pulmonary hypertension piglet model consisting of left pulmonary artery ligation and repeated embolisation in the right lower lobe: bronchial artery hypertrophy in the ischemic lung territory (right close-up), apparently normal right upper lobe (left close-up), unresolved intravascular material made of tissue adhesive and fibrin moulding the arterial tree, and right ventricle remodeling.

**Table 1:** Insights on thrombus resolution and organisation learned from stagnant flow induced inferior vena cava thrombus animal models

**Table 2:** Current animal models used to study pathogenesis and pathobiology of chronic thromboembolic pulmonary hypertension
<table>
<thead>
<tr>
<th>Animal Models</th>
<th>Species</th>
<th>Strengths</th>
<th>Weaknesses</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVC ligation or stenosis models (day 0 to day 28)</td>
<td>Rodents, pig, primate</td>
<td>Reproduce thrombus resolution and organisation</td>
<td>- Studies on vein (not PA)</td>
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<td></td>
<td></td>
<td></td>
<td>- No PH</td>
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<td></td>
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<td></td>
<td>- No additional vasculopathy</td>
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<tr>
<td>[7-24, 27]</td>
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<tr>
<td>PA ligation models (5 weeks)</td>
<td>Rodents, pig</td>
<td>Reproduce obstructed territories with postobstructive vasculopathy (bronchial circulation, distal pulmonary vasculopathy)</td>
<td>- No pathogenesis</td>
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<tr>
<td></td>
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<td>- No PH</td>
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<td></td>
<td></td>
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<td>- No RV remodeling and dysfunction</td>
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<td></td>
<td></td>
<td></td>
<td>- No distal vasculopathy in non-obstructed lung</td>
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<tr>
<td>[29-40]</td>
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<tr>
<td>Pulmonary overflow models (aorto-pulmonary shunt – 5 weeks)</td>
<td>Pig</td>
<td>Reproduce non-obstructed territories with distal pulmonary vasculopathy with media thickness</td>
<td>- No pathogenesis</td>
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<tr>
<td></td>
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<td>- No post obstructive vasculopathy</td>
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<td>- No pulmonary obstruction</td>
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<td>[41-45]</td>
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<tr>
<td>PA ligation + glue embolisation (5 weeks)</td>
<td>Pig</td>
<td>- Reproduce obstructed and non obstructed territories</td>
<td>- No pathogenesis</td>
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<tr>
<td>[53]</td>
<td></td>
<td>- Reproduce RV remodeling and dysfunction</td>
<td>- Reproduce sustained PH</td>
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Table 2
<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>Animals</th>
<th>Models</th>
<th>Hypothesis</th>
<th>Conclusions</th>
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<tr>
<td>Fowkes J and al (17)</td>
<td>1992</td>
<td>rat and primate</td>
<td></td>
<td>IVC thrombosis by ligation or balloon occlusion</td>
<td>Thrombus ultrasound echogenicity could predict clot age in two different animal species</td>
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<td>Kney C and al (12)</td>
<td>2002</td>
<td>pig</td>
<td></td>
<td>Co-encapsulation with 30% or 50% of gelatin was rapidly replaced by a vascular prosthesis aortic luminal vascular prostheses: a thrombogenesis induction</td>
<td>Description of a new animal model of vascular thrombus mimicking human disease.</td>
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<td>Vla H and al (75)</td>
<td>1994</td>
<td>rat</td>
<td>IVTh</td>
<td>IVTh thrombosis by ligation</td>
<td>Transtibial ultrasound elasticity could predict clot age.</td>
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**Inflammation and Thrombus resolution**

<table>
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<tr>
<th>Authors</th>
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<tr>
<td>Wakefield T and al (24)</td>
<td>1995</td>
<td>rat</td>
<td>IVTh</td>
<td>Inflammation into the vein wall during thrombosis, extravasation of neutrophils and monocyte/macrophage: role of cytokines</td>
<td>Neutrophil accumulation (TNF-α, NFkB), IL-8, MCP-1; monocyte/macrophage infiltration (MCP-1, NFkB, IL-8) increased during thrombus formation.</td>
</tr>
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<td>Wakefield T and al (19)</td>
<td>1999</td>
<td>rat</td>
<td>IVTh</td>
<td>Role of IL-8 in thrombus resorption</td>
<td>III B augmented thrombus resorption</td>
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<tr>
<td>Landy F and al (15)</td>
<td>2004</td>
<td>rat</td>
<td>IVTh</td>
<td>Role of IL-8 in inducing persistent inflammation associated with HMG</td>
<td>MRI could quantify venous inflammation which is modified by IL10 treatment</td>
</tr>
<tr>
<td>Horis P and al (22)</td>
<td>2001</td>
<td>mice</td>
<td>IVTh</td>
<td>Inflammation and thrombus resolution: role of neutrophil and its receptor Cxcr2</td>
<td>Thrombus resolution involved CXCR2 pathway.</td>
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<td>Vaarea M and al (21)</td>
<td>2003</td>
<td>rat</td>
<td>IVTh</td>
<td>Role of neutrophils in thrombus resolution</td>
<td>Neutrophils altered thrombotic activity slowing down thrombus resolution and increased thrombin collagen.</td>
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<td>Henke P and al (26)</td>
<td>2001</td>
<td>rat</td>
<td>IVTh</td>
<td>Role of IL8 (proinflammatory and proangiogenic cytokine) in thrombus resolution</td>
<td>IL8 enhanced thrombus resolution and increased remodeling by improving thrombus necrosis and increasing neutrophil.</td>
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<td>Singh J and al (14)</td>
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<td>mice</td>
<td>IVTh</td>
<td>Bids of IL-8 and IL-10 in thrombus resolution</td>
<td>Bids of IL-8 and IL-10 in thrombus resolution</td>
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<td>Hummers J and al (13)</td>
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<td>IVTh</td>
<td>IVTh thrombosis by 60-90% external stent</td>
<td>Injection of MCP-1 in thrombus resolution:</td>
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<td>IVTh</td>
<td>IVTh thrombosis by 60-90% external stent</td>
<td>Injection of MCP-1 in thrombus resolution:</td>
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<td>Nestorovskij and al (77)</td>
<td>1998</td>
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<td>Role of neutrophils and macrophages in thrombus resolution</td>
<td>Neutrophils migrated into the thrombus during resolution</td>
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<td>Vanhoutte P and al (67)</td>
<td>1998</td>
<td>mice</td>
<td>IVTh</td>
<td>IVTh thrombosis by 60-90% external stent</td>
<td>IVTh thrombosis injection enhanced fibrinolytic activity resulting from thrombus-macrophage interaction.</td>
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**Angiogenesis and Thrombus resolution and organisation**

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<td>Waltham M and al (12)</td>
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<td>Role of VEGF and bFGF in thrombus resolution</td>
<td>VEGF and bFGF had temporal expression patterns during thrombus resolution and organisation</td>
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<td>2003</td>
<td>rat</td>
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<td>Role of VEGF in thrombus resolution</td>
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<td>Waltham M and al (9)</td>
<td>2005</td>
<td>rat</td>
<td>IVTh</td>
<td>Effect of VEGF gene transfer after thrombus resolution</td>
<td>Human VEGF gene plasmid transfer into the thrombus enhanced thrombus resolution and recanalization with macrophage recruitment</td>
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<tr>
<td>Mehnert R and al (4)</td>
<td>2008</td>
<td>rat and mice</td>
<td>IVTh</td>
<td>Effect of Adenovirus-mediated ARF gene therapy on thrombus resolution</td>
<td>Injection of Adenovirus-mediated VEGF gene therapy enhanced thrombus resolution and recanalization with macrophage recruitment</td>
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